

FILE 'MEDLINE, CAPLUS' ENTERED AT 12:05:18 ON 29 OCT 2003

L1 109 S (DILUT? (P) ENZYME? (P) STORAGE)
L2 108 DUP REM L1 (1 DUPLICATE REMOVED)
L3 2 S L2 AND POLYMERASE?
L4 49 S (DILUT? (P) POLYMERASE? (P) (STABLE OR STABILITY))
L5 49 DUP REM L4 (0 DUPLICATES REMOVED)
L6 16 S (DILUT? (P) POLYMERASE (P) (STORAGE OR STORE))
L7 16 DUP REM L6 (0 DUPLICATES REMOVED)

=>

L7 ANSWER 6 OF 16 MEDLINE on STN
 AN 96387496 MEDLINE
 DN 96387496 PubMed ID: 8795005
 TI Factors affecting detection of PVY in dormant tubers by reverse transcription polymerase chain reaction and nucleic acid spot hybridization.
 AU Singh M; Singh R P
 CS Agriculture and Agri-Food Canada, Fredericton Research Centre, New Brunswick, Canada.
 SO JOURNAL OF VIROLOGICAL METHODS, (1996 Jun) 60 (1) 47-57.
 Journal code: 8005839. ISSN: 0166-0934.
 CY Netherlands
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199612
 ED Entered STN: 19970128
 Last Updated on STN: 19970128
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 AB A reverse transcription **polymerase** chain reaction (RT-PCR) protocol was developed using two 20-mer primers located in nuclear inclusion genes NIa and NIb of potato virus Y (PVY). A 1017 bp PCR-product was detected in dormant potato tubers, infected with PVY(O), but not in tubers from healthy plants. The PCR product was specific to PVY, as determined by Southern blot detection by hybridization with a PVY(O)-specific probe. As little as 1 pg of purified PVY(O)-RNA can be detected after RT-PCR amplification. The presence of phenolics or polysaccharides in tuber nucleic acids inhibited PVY(O) amplification, which was eliminated by **diluting** nucleic acid preparations prior to cDNA synthesis, modifying the nucleic acid extraction procedure by isopropanol precipitation and using phosphate-buffered saline-Tween in the cDNA mix. Potato cultivars differed in PVY(O) concentration in tubers as much as 128-fold. Tuber parts used for nucleic acid extractions were important in potato cultivars with low virus titres and did not result in reduced detection of PVY(O) by both nucleic acid spot hybridization and RT-PCR, but RT-PCR band intensity was lower at longer **storage** periods. The primer pair developed in this study exhibited broad specificities with field isolates from Peru, Scotland and North America.